175* Histone deacetylases (HDACs) and IFRD1 in CF airway epithelial cell models

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In cystic fibrosis (CF) patients, recent data suggest that Interferon related developmental regulator 1 (IFRD1) is a modifier gene for lung disease. IFRD1 acts in a histone-deacetylase (HDAC)-dependent manner to mediate transcriptional co-repression of NF-κB transactivation involved in inflammation. We therefore hypothesize that intrinsic alterations might occur in the balance of HDAC1–3 and IFRD1 expression in CFTR-deficient cells compared to normal and corrected CF airway cells.

We have examined the expression level of three HDAC1,2,3 mRNA and IFRD1 mRNA (by qPCR) in two CF bronchial cell lines (IB3–1 and CFBE41o–) compared to CFTR-corrected and sufficient (S9 and 16HBE14o–) cell models. We also evaluated the level of HDACs1–3 proteins by Western blotting and measured the total HDACs enzymatic activity under oxidative stress (IL-1β, 10 ng/ml and/or H_2O_2, 100 and 500 μM, 1 h).

First, we show a lowest expression of HDACs(1–3) and IFRD1 mRNA in CFBE41o– cells compared to 16HBE14o– cells. We observe that the level of HDACs(1–3) proteins and particularly HDAC2 is reduced in CFTR-deficient cells compared to CFTR-sufficient cells in response to oxidative stress. We also demonstrate that total HDAC activity was lower in IB3–1 and CFBE41o– cells compared to S9 and 16HBE14o– cells at both basal and oxidative stress conditions. Together, our data show significant difference in the HDAC1–3/IFRD1 signaling in CFTR deficient cells compared to normal and corrected CF airway cells. Understanding how loss of CFTR function leads to alterations in the regulation of IFRD1/HDACs complex and its role in exaggerated inflammatory response in CF airway cells require further investigation.

176* Altered expression of T-cell immunoglobulin and mucin-domain-containing molecule-1 (TIM-1) and TIM-3 in the cystic fibrosis airway

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Background: The T-cell immunoglobulin and mucin-domain containing molecules (TIMs) constitute a new receptor protein family involved in regulation of immune responses. However, the role of TIM receptors in cystic fibrosis (CF) is unknown. The aim of this study was to examine the expression of TIM receptors in the CF airway epithelium.

Methods: We examined the expression of TIM-1 and TIM-3 in CF airway epithelial cells using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), laser scanning microscopy and Western blotting. Cell surface expression of TIM-3 was confirmed by membrane surface biotinylation and immunofluorescence confocal microscopy. We also investigated the effect of the pro-inflammatory stimuli, lipopolysaccharide (LPS), on TIM expression.

Results: Our results demonstrate that TIM-1 and TIM-3 are expressed in human bronchial epithelial cells. We also show that TIM-1 and TIM-3 are significantly upregulated in quiescent CF cells, implying a role for the cystic fibrosis transmembrane conductance regulator channel in TIM expression. Finally, our experiments revealed that TIM-3 expression can be modulated by pro-inflammatory stimuli. LPS treatment induced a 3-fold increase in TIM-3 expression in CF cells versus 2-fold increase in controls ($p < 0.001$).

Conclusions: Our work reports for the first time, the expression of TIM receptors in human bronchial epithelial cells. The upregulation of TIM-1 and TIM-3 may represent a novel intrinsic defect that contributes to the aberrant immune response in the CF airway.

177 Both type II (T2SS) and type III (T3SS) secretion systems of *Pseudomonas aeruginosa* play roles in death due to lung disease

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The T3SS of *P. aeruginosa* (*P.a.*) is recognized to be an important cause of death during acute lung infection. However, the role of T2SS in this process has not been conclusively demonstrated. Using TLR2,4 –/– mutant mice and flagellin negative mutant strains of *P.a.*, essentially abrogating the major innate immune responses against LPS and flagellin, we unequivocally demonstrate that these toxin secretion systems play redundant roles in death from acute lung infections. We challenged TLR2,4 –/– mice with T2 and T3SS mutants of strain PAK with a flagellin gene mutation. Mice were observed for mortality or sacrified to measure host inflammatory responses and bacterial counts and for histological studies. Strains having only T3SS killed mice within 24 h. Strains with only the T2SS also killed mice, but with a delay. Strains lacking both secretion systems were avirulent. Histological studies demonstrated a delayed onset of pathological lesions that progressed with time, when the T2SS was the sole system present. Minimal inflammation, which did not progress, was noted when both secretion systems were absent. In the absence of both secretion systems the mice cleared the microorganisms in 44 h, by contrast when the T2SS was intact, bacterial counts at 37–44 h had increased. These data demonstrate that the T2SS of *P.a.* also plays a role in death due to lung infections, and that this system secretes factors that impair bacterial clearance, besides causing death. This work also suggests that in this strain of *P.a.* and this animal model, the non T2/T3SS do not contribute significantly to death.

178 Association of MASP3 and FCN2 gene polymorphisms with the age of onset of chronic *Pseudomonas aeruginosa* (*Pa*) colonization in cystic fibrosis (CF)

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Background: Modifying genes of innate immunity may be involved in the early onset of chronic *Pa* colonization (C*Pa*C) in CF.

Methods: 82 Single Nucleotide Polymorphisms (SNPs) in 22 genes contributing to innate immunity [MBL2, MASP (MBL-associated serine Protease)1/2/3, FCN (Ficolin)1/2, LBP (Lipopolysaccharide-binding Protein), CD14, TLR (Toll-like receptors $1 \rightarrow 10$)] were genotyped in an initial cohort of 116 CF patients and later expanded to 215 CF patients. The frequency of SNPs was compared between 2 subgroups: C*Pa*C patients versus non colonized patients and C*Pa*C patients colonized before/or at the age of 8, 10 and 12 years (y) versus C*Pa*C patients colonized older than 8, 10 and 12 y.

Results: In C*Pa*C patients an increased frequency of polymorphism –1981(A>G) **FCN1** (promoter) (Odds ratio [OR] = 2.87 [95% confidence interval (CI): 1.21–6.8] p = 0.01) and polymorphism Q275Q (G>A) **FCN1** (OR = 3.31 [1.40–7.85] p = 0.005) was found compared to non *Pa* colonized patients. Looking at the age of onset of *Pa* colonization, L617L(G>A) polymorphism of **MASP3** (OR = 4.38 [1.37–14.02] p = 0.009), –64A>C polymorphism **FCN2** (promoter) (OR = 5.63 [1.63–19.42] p = 0.003) and S258A(X>Y) polymorphism **FCN2** (OR = 6.62 [1.91–22.89] p = 0.001) were significantly more common in C*Pa*C patients colonized before/or at 10 y compared to those colonized older than 10 y. In the larger group, S258A in FCN2 remained more common in C*Pa*C patients colonized younger than or equal to 10 y (OR = 3.2 [1.22–8.46] p = 0.01).

Currently, we are performing Multifactor Dimensionality Reduction for detecting interactionsin SNPs.

Conclusion: Polymorphisms L617L of MASP3,–64A>C and S258A of FCN2 are significantly more common in C*Pa*C patients colonized at a younger age.